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U.S. Army Center for Health Promotion and Preventive Medicine
(Provisional)

TOXICOLOGICAL STUDY NO. 75-51-Y2Z6-95
THE ACUTE TOXICITY OF A MIXTURE OF THE INSECT REPELLENTS DEET AND AI3-37220
MAY 1995

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U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE (Provisional)

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) lineage can be traced back over fifty years to the Army Industrial Hygiene Laboratory. That organization was established at the beginning of World War II and was under the direct jurisdiction of The Army Surgeon General. It was originally located at the Johns Hopkins School of Hygiene and Public Health, with a staff of three and an annual budget not to exceed three thousand dollars. Its mission was to conduct occupational health surveys of Army operated industrial plants, arsenals, and depots. These surveys were aimed at identifying and eliminating occupational health hazards within the Department of Defense's (DOD) industrial production base and proved to be beneficial to the Nation's war effort.

Most recently, it has been nationally and internationally known as the U.S. Army Environmental Hygiene Agency or AEHA. Its mission, by this time, had been expanded to support the worldwide preventive medicine programs of the Army, DOD and other Federal Agencies through consultations/supportive services, investigations and training.

Today, it is redesignated the U.S. Army Center for Health Promotion and Preventive Medicine. Its mission for the future is to provide worldwide technical support for implementing preventive medicine, public health and health promotion/wellness services into all aspects of America's Army and the Army Community anticipating and rapidly responding to operational needs and adaptable to a changing world environment.

The professional disciplines represented at the Center include chemists, physicists, engineers, physicians, optometrists, audiologists, nurses, industrial hygienists, toxicologists, entomologists, and many others as well as sub-specialties within these professions.

The organization's quest has always been one of excellence and continuous quality improvement; and today its vision, to be the nationally recognized Center for Health Promotion and Preventive Medicine, is clearer than ever. To achieve that end, it holds ever fast to its values which are steeped in its rich heritage:

- Integrity is the foundation
- Excellence is the standard
- Customer satisfaction is the focus
- Its people are the most valued resource
- Continuous quality improvement is its pathway

Once again, the organization stands on the threshold of even greater challenges and responsibilities. It is being totally reorganized with a provisional structure and will obtain its first General Officer leadership. As it moves into the next century, new programs are being added related to health promotion/wellness, soldier fitness and disease surveillance. As always, its mission focus is centered upon the Army Imperatives so that we are trained and ready to enhance the Army's readiness for war and operations other than war.

It is an organization fiercely proud of its history, yet equally excited about the future. It is destined to continue it development as a world-class organization with expanded services to the Army, DOD, other Federal Agencies, the Nation and the World Community.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Distribution unlimited; approved for public release.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) 75-51-Y2Z6-95	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION U.S. Army Center for Health Promotion & Preventive Med. 6b. OFFICE SYMBOL (*If applicable*) MCHB-DL-TE	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (*City, State, and ZIP Code*) Aberdeen Proving Ground, MD 21010-5422	7b. ADDRESS (*City, State, and ZIP Code*)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Material Activity 8b. OFFICE SYMBOL (*If applicable*) SGRD-UMB	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (*City, State, and ZIP Code*) Fort Detrick, Frederick, MD 21702-5009	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (*Include Security Classification*) Toxicological Study No. 75-51-Y2Z6-95, The Acute Toxicity of a Mixture of the Insect Repellents Deet and AI3-37220, May 1995
UNCLASSIFIED

12. PERSONAL AUTHOR(S)
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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (*Year, Month, Day*)	15. PAGE COUNT
FINAL	FROM 2/94 TO 9/94	95 05 01	32

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

The purpose of the study was to determine the toxicity to animals of a mixture of the insect repellents Deet and AI3-37220, and to assess its dermal effects in humans. A mixture of the insect repellents Deet and AI3-37220 in 95 percent alcohol (25:25:50) did not cause primary skin irritation nor sensitization in animals, was moderately toxic by the oral route but was essentially nontoxic dermally. In humans, no skin irritation nor sensitization was produced by the repellents mix. The toxicity of the repellents mixture did not exceed the toxicities of the individual components. It is recommended that repellents mixtures containing up to 25 percent each of Deet and AI3-37220 undergo advanced entomological testing in humans. Based upon the known eye irritation potential of the individual components, it is recommended that mixtures of Deet and AI3-37220 in ethyl alcohol be used with caution around the eyes and mucosa.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Hubert L. Snodgrass	22b. TELEPHONE (*Include Area Code*) (410) 671-3980 22c. OFFICE SYMBOL MCHB-DL-TE

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

Sponsor

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Study Title

The Acute Toxicity of a Mixture of the Insect Repellents
Deet and AI3-37220

Data Requirement

Author

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Study Completed On

1 September 1994

Performing Laboratory

U.S. Army Center for Health Promotion and Preventive Medicine (Provisional)
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Laboratory Project ID

75-48-Y2Z6-94

U. S. Center For health Promotion and Preventive Medicine
Quality Assurance Office

Quality Assurance Statement For: Toxicology Study Number 75-51-Y2Z6-94, The Acute Toxicity of a Mixture of the Insect Repellant Deet and AI3-37220.

Critical Phase Audited	Date Audited	Date Reported to Mgmt.
Photochemical Skin Irritation	15 Feb 95	18 Feb 95
Primary Skin Irritation	1 Mar 95	3 Mar 95
Study Laboratory Notebook	3 Mar 95	3 Mar 95
Approximate Lethal Dose (Oral)	5 May 94	9 May 94
Final Report	17 Jul 95	19 Jul 95

GENE SINAR
GLP Assessor, Quality Assurance Office

Toxicological Study No. 75-51-Y2Z6-95, May 95

Study Compliance Statement

The animal studies described in this report were conducted in compliance with Title 40 Code of Federal Regulations (CFR) Part 792, Good Laboratory Practice Standards. There were no deviations from the aforementioned regulations except those listed below:

1. Primary skin irritation study in rabbits - Animals exceeded the weight range specified in the SOP.

2. Photochemical skin irritation study in rabbits - Animals exceeded the weight range specified in the SOP.

3. Approximate lethal dose procedures - Animals exceeded the weight range specified in the SOP.

The deviations did not effect the quality or integrity of the study.

LeRoy W. Metker

LEROY M. METKER
Study Director

Date 26 July 95

Toxicological Study No. 75-51-Y2Z6-95, May 95

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Date 26 July 95

DEPARTMENT OF THE ARMY
U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE (PROVISIONAL)
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
ATTENTION OF

EXECUTIVE SUMMARY
TOXICOLOGICAL STUDY NO. 75-51-Y2Z6-95
THE ACUTE TOXICITY OF A MIXTURE OF THE INSECT REPELLENTS DEET AND AI3-37220
MAY 1995

1. PURPOSE. The purpose of the study was to determine the toxicity to animals of a mixture of the insect repellents Deet and AI3-37220 and to assess its dermal effects in humans.

2. CONCLUSIONS. A mixture of the insect repellents Deet and AI3-37220 in 95 percent alcohol (25:25:50) did not cause primary skin irritation in animals. The mixture was moderately toxic by the oral route but was essentially nontoxic dermally. No skin sensitization was demonstrated in animals. In humans, no skin irritation nor sensitization was produced by the repellents' mix. The toxicity of the repellents' mixture did not exceed the toxicities of the individual components. Accordingly, neither additive nor synergistic effects were demonstrated.

3. RECOMMENDATIONS.

a. Based upon the results of toxicity studies in animals, and in humans, it is recommended that the repellents' mixtures containing up to 25 percent each of Deet and AI3-37220 undergo advanced entomological testing in humans.

b. Based upon the known eye irritation potential of the individual components, it is recommended that mixtures of Deet and AI3-37220 in ethyl alcohol be used with caution around the eyes and mucosa.

Toxicological Study No. 75-51-Y2Z6-95, May 95

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DEPARTMENT OF THE ARMY
U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE (PROVISIONAL)
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
ATTENTION OF

MCHB-DL-TE

TOXICOLOGICAL STUDY NO. 75-51-Y2Z6-95
THE ACUTE TOXICITY OF A MIXTURE OF THE INSECT REPELLENTS DEET AND AI3-37220
MAY 1995

1. REFERENCES. See Appendix A for a listing of references.

2. AUTHORITY. Letter, U.S. Army Medical Material Activity, 28 Dec 1993, subject: Animal Toxicity Testing of Insect Repellents.

3. PURPOSE. The study was conducted to determine the acute toxicity of a mixture of the insect repellents Deet and AI3-37220 in animals, and its skin effects in humans.

4. BACKGROUND.

a. The use of topical insect repellents by military personnel is often their only protection against insect-borne diseases. Repellents containing Deet are the standard issue military items. Deet is also the most common active ingredient in consumer-use products. No repellent, however, is equally effective against all insect species. It has been proposed that by combining two (or more) substances, a repellent with a wider spectrum of effectiveness may be produced.

b. AI3-37220 has been tested for insect repellency by the U.S. Department of Agriculture and found to equal or exceed the efficacy of Deet (reference 1). The toxicity of AI3-37220 was evaluated under the Topical Hazard Evaluation Program (THEP) by the U.S. Army Environmental Hygiene Agency (USAEHA) (reference 2). Results from the THEP, and later toxicity studies conducted at USAEHA (reference 1), showed that the material presented no serious toxicological concerns. Accordingly, USAEHA recommended continued exploratory development of the topical insect repellent.

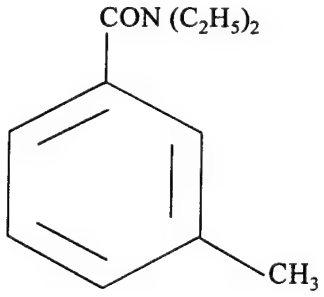
Use of trademarked names does not imply endorsement by the U.S. Army but is intended only to assist in identification of a specific product.

c. The present studies were performed to determine if there was any interaction between Deet and AI3-37220 that would increase the toxicity of the mixture as measured in animals. The proportion of the ingredients, e.g., 25 percent each, represented the maximum expected concentration of a final product. A prophetic patch test in humans measured dermal effects of the mixture using skin sensitization as an endpoint. A 21-day cumulative irritancy assay, also performed in humans, measured the effects on the skin to repeated applications of the mixture.

5. MATERIALS.

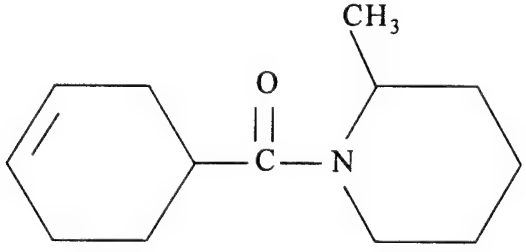
a. Test Substances.

(1) Deet (CAS No. 134-62-3) was provided by Morflex Incorporated, Greensboro, North Carolina. It had a measured meta isomer content of 98.6 percent. It was identified as Lot No. N36240. Its structure and chemical name are as follows:



N,N-diethyl-m-toluamide

(2) AI3-37220 (CAS No. 77251-47-9) was purchased from Daychem Chemical Company, Dayton, Ohio and redistilled by S.C. Johnson Wax, Racine, Wisconsin. It was identified as Lot No. SCJ-1 and had a measured chemical purity of >99 percent. Its structure and chemical name are as follows:



1-(3-Cyclohexen-1-ylcarbonyl)-2-methylpiperidene

(3) Ethyl alcohol, 200 proof, USP, was purchased from Midwest Grain Products Company, Weston, Missouri. It was marked Lot No. DSP-MO-5. The alcohol was diluted to 95 percent with distilled water. This solution was the vehicle for the all toxicity testing of the repellents mixture except where noted.

(4) For most testing, a mixture of AI3-37220 and Deet was combined with 95 percent ethyl alcohol to a 25:25:50 ratio by volume. In one of the oral toxicity studies in rats, the neat repellents were used (no alcohol) resulting in a 50:50 mixture by volume.

b. Animals[1,2].

(1) Tests for primary skin irritation and photochemical skin irritation were conducted using New Zealand White rabbits of mixed sex weighing between 3.6 and 4.9 kg. Rabbits were purchased from Hazelton-Dutchland Laboratories, Denver, Pennsylvania. Male and female Sprague-Dawley rats, weighing 260-315 g and 185-220 g, respectively, were used in the oral toxicity studies. Rats were purchased from Charles River Laboratories, Wilmington, Massachusetts. Dermal toxicity and skin sensitization studies were conducted using Albino-Hartley guinea pigs of mixed sex and weighing 400-500 g. Guinea pigs were also purchased from Hazelton-Dutchland Laboratories.

(2) Rabbits, guinea pigs and rats were housed individually in wire-bottom stainless steel cages. Drinking quality water and feed (Purina® Certified Rabbit Chow 5322; Purina Certified Guinea Pig Chow 5025; and Purina Certified Rodent Chow 5002) were available *ad libitum*. Ambient temperatures in the animal rooms were maintained at 21 to 25 °C with relative humidity between 40 and 60 percent. The light/dark cycle was a 12-hour interval.

c. Contract Studies.

(1) A 21-Day Cumulative Irritancy Assay was conducted in humans under contract No. DAAD05-94-P-1082 (Study #HIM 94-M-I-1) by Howard I. Maibach, M.D., San Francisco, California. Ten adult subjects (over 18 years of age) made up the test panel. All subjects were examined and deemed free of any active skin pathology. Medical histories and consent forms were obtained from all subjects.

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[1] In conducting the studies described herein, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health Education and Welfare Publication No. (NIH) 85-23, 1985.

[2] The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

(2) A Modified Draize Skin Sensitization Study was conducted in humans under contract No. DAADO5-94-P-1082 (Study #HIM 94-USAEHA-D-1) by Howard I. Maibach, M.D., San Francisco, California. The test panel included 200 adult subjects. They were examined prior to commencement of the study and deemed to be free of any active skin pathology. Medical histories and consent forms were obtained from all subjects.

6. METHODS.

a. Primary Skin Irritation. An acute dermal irritation test was conducted using rabbits. The procedure (reference 3) involved the single application of 0.5 mL of the test mixture (Deet and AI3-37220 in ethyl alcohol, 25:25:50) to the clipped backs of each of six rabbits. The material was placed on a 2X2 inch gauze pad, applied to the skin surface and then over-wrapped with an occlusive covering. Exposure was for 24 hours, after which the coverings were removed and irritation scored 1 hour later. Evaluations were also made at 48 and 72 hours and again at 7 days. Scoring of irritation was based on the Draize method in which erythema and edema were evaluated on a scale of 0 to 4 for severity. Categorizing the responses was based upon the mean of the 24- and 72-hour scores.

b. Photochemical Skin Irritation. Studies were performed (reference 4) by applying 0.05 mL of the test mixture (25:25) to the right side of the clipped backs of six rabbits. After 5 minutes, the backs were exposed to ultraviolet (UV) light irradiation (365 nm). The exposure times varied according to the irradiance produced by the calibrated light sources. The left side of the back was light-protected during the exposure. Oil of Bergamot (10 percent in 80 percent ethanol), a known photo irritant, was included as a positive control to assure the responsiveness of the test system. Following UV exposure, the left side of the same animal's back was treated identically to the right, except that it was not irradiated. Scoring of erythema and/or edema was based upon the method of Draize. Photochemical irritation, as determined by the net difference between irradiated and nonirradiated scores, was evaluated at 24, 48, and 72 hours.

c. Skin Sensitization. The test procedure was based upon the method of Buehler (reference 5). It is used to predict the possible delayed contact hypersensitivity to a chemical. The test (reference 6) was conducted using 20 guinea pigs, each receiving a single dermal application of the test mixture once a week for 3 weeks. The substance (0.3 mL) was applied under an occlusive patch (Webril®) for each 6-hour exposure period. A minimally irritating dose, based on preliminary testing, was used. It was a 5 percent concentration (w/v) of the 25:25 mixture, diluted with 80 percent ethyl alcohol. Following a

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13-day rest period, each animal was challenged with a dermal patch containing 0.3 mL of the test mixture at a previously determined nonirritating level. The nonirritating concentration of the 25:25 mixture was 1 percent (w/v). The diluent was 80 percent ethyl alcohol. Dermal reactions to the challenge were compared to the responses of ten naive guinea pigs who received only the single challenge dose.

d. Oral Toxicity. The toxicity of the repellents' mixture (25:25) was measured in five male and five female rats at a single high limit dose of 2,000 mg active ingredient (A.I.)/kg (reference 7). An additional 10 animals were treated with a 50:50 mixture (no alcohol vehicle) of the two repellents. Rats were treated by gavage. Observations of toxic signs were made daily through 14 days. Survivors were necropsied at day 14 and examined for gross pathological changes.

e. Dermal Toxicity. A single high limit dose of the mixture (25:25), 2,000 mg A.I./kg, was applied to the clipped backs of five guinea pigs (reference 7). The test material was injected under an occlusive rubber sleeve which surrounded the clipped trunk of each animal. The sleeve was removed after 24 hours. Animals were examined daily for toxic signs through 14 days.

f. 21-Day Cumulative Irritancy Assay (in humans). The procedure is that summarized by Phillips (reference 8). The test mixtures were applied to the skin under an occlusive plastic chamber (Hilltop®) 5 days weekly for 21 days to the same site. They were not reapplied on weekends (or holidays) but remained in place for these periods. There were 15 days of readings, even when holidays intervened. Readings for skin irritation were made at each removal of the covering on a scale of 0-4. The mixtures and control articles tested were:

(a) 25 percent Deet and 25 percent AI3-37220 in ethyl alcohol*

(b) 12.5 percent Deet and 12.5 percent AI3-37220 in ethyl alcohol*

(c) 6.25 percent Deet and 6.25 percent AI3-37220 in ethyl alcohol*

(d) 3.125 percent Deet and 3.125 percent AI3-37220 in ethyl alcohol*

(e) 25 percent Deet in ethyl alcohol*

® Hilltop is a registered trademark of Hilltop Laboratories, Cincinnati, Ohio.
* The ethanol concentration was 95 percent in all cases.

(f) 100 percent ethyl alcohol*

(g) 25 percent AI3-37220 in ethyl alcohol*

g. Skin Sensitization Study (in humans). The study was a modification of the procedure set forth by Draize (reference 9). Test patches were moistened with approximately 0.2 g of the test material. The patch was an occlusive plastic chamber (Hilltop) held in place with paper tape (Scanpore, Norgeplaster, Oslo, Norway). Patches of the test materials were applied to the upper arms or backs of all panelists. All applications of samples were made to the same site. The study was performed in approximately a 6-week period for each subject. During the first 3 weeks, or the induction period, patches were applied thrice weekly for 48-72 hours. The panelists were instructed to leave the patches on and keep them dry following each application. Approximately 2 weeks after the induction (sensitization) phase, the challenge applications were made. The patch was applied to a previously unpatched site. The challenge patches were removed 72 hours following applications. Reactions to the challenge applications were scored at 96 hours following the challenge. The mixtures tested were as follows:

(a) 3.125 percent Deet and 3.125 percent AI3-37220 in ethyl alcohol (95 percent).

(b) 25 percent Deet and 25 percent AI3-37220 in ethyl alcohol (95 percent).

(c) 100 percent AI3-37220.

7. RESULTS.

a. Primary Skin Irritation. The test mixture, Deet and AI3-37220 (25:25) in ethyl alcohol, did not produce primary irritation to the skin of rabbits. No effects were noted in any of the animals when observed at 24 and 72 hours following exposure, nor at 7 days. Individual animal data appears in Appendix B.

b. Photochemical Skin Irritation. The test mixture did not cause a photochemical skin irritation response in rabbits. The net difference between UV-irradiated and nonirradiated exposure sites was not significant. The net total score was 0.16. (A total score of 1.0 or higher is considered a positive reaction.) See Appendix C for individual scores. Oil of Bergamot, the positive control substance tested in the same animals, cause a significant increase in irritation when irradiated. The net score was 1.22. See Appendix D for individual animal data.

c. Skin Sensitization. The test mixture did not produce skin sensitization in guinea pigs under the conditions of the test protocol. The individual animal data appears as Appendix E.

d. Oral Toxicity. At the limit dose of 2,000 mg A.I./kg, no deaths were observed in male rats treated with a single oral dose of the test mixture (25:25) or a 50:50 mixture of Deet and AI3-37220. Accordingly, the predicted LD_{50} for either mixture in male rats is >2,000 mg A.I./kg. In female rats treated orally with the 25:25 mixture, two of five animals died within 24 hours of treatment at 2,000 mg A.I./kg. Using the 50:50 mixture, one of five females died 48 hours after oral treatment. The predicted LD_{50} for either mixture in female rats is ≥2000 mg A.I./kg. Nonfatal toxic signs observed in both male and female rats were lethargy and ataxia. Lethargy was noted in all rats treated with the 25:25 mixture but was essentially absent in rats receiving the 50:50 mix. No treatment-related gross pathological abnormalities were observed in any of the rats necropsied 14 days after treatment. Individual male rat data appears as Appendices F and H; female rat data appears as Appendices G and I.

e. Dermal Toxicity. The test mixture (25:25) applied dermally to guinea pigs did not produce toxicity at the 2,000 mg A.I./kg dose level. No toxic signs resulted from the 24-hour occlusive exposure. See Appendix J for individual animal data.

f. 21-Day Cumulative Irritancy Assay. Repeated applications of varying mixtures of Deet and AI3-37220, up to 25 percent of each, produced only minimal skin effects in humans during 15 applications to the same site. Six of the ten panelists had no observable responses to the repellents. In the remaining subjects, most scores were 0.5, indicating an equivocal response. Some progressed to grade 1.0, or minimal erythema. This (1.0) was the highest score observed. The highest cumulative scores were reported for the nonmixtures 25 percent Deet in ethanol (score 19.5) and for 25 percent AI3-37220 (score 21.5). The alcohol vehicle alone produced a cumulative score of 7.0. A summary of the cumulative irritant scores appears at Appendix K.

g. Skin Sensitization Study. There was no evidence of the induction or elicitation of allergic contact dermatitis in 200 human subjects treated with neat AI3-37220 or a 25:25 mixture of Deet and AI3-37220 in 95 percent ethyl alcohol.

8. DISCUSSION.

a. The Table presents data on the toxicity of the components of the repellents mixture. It is apparent that no increase in toxicity would be expected as a result of mixing Deet and AI3-37220 in ethyl alcohol over that of the individual components. Dermal and oral toxicity results were comparable to data provided for either Deet or AI3-37220 when total mass active ingredient was considered. No skin irritation or sensitization would be anticipated in humans at the levels up to 25 percent for each repellent component (50 percent total A.I.).

b. Eye irritation studies were not performed in animals using the repellents mixture. Both Deet and AI3-37220 produce mild to moderate eye irritation as noted in the Table. Ethyl alcohol, the diluent for the test mixture, causes moderate to severe eye irritation following acute exposures (see the Table). Accordingly, the mixture should be used with caution around the eyes and mucosa.

TABLE. COMPARATIVE TOXICITIES OF DEET, AI3-37220 AND ETHYL ALCOHOL TO A 25:25:50 MIXTURE OF THE THREE (references 10, 11, and 12)

Study	Speci	Deet	AI3-37220	Ethyl alcoh	Mixture
Prim Skin Irrit	Rab	500 mg mild	500 mg mild	20 mg mod	500 mg neg
Prim Eye Irrit	Rab	10 mg mod 100 mg mod	100 mg mild	500 mg mild to sev	Not perfrmd
Photochem Irrit	Rab	Neg	Neg	-----	Neg
Skin Sensitiz	GPg	Neg	Neg	Neg	Neg
Acute Oral LD_{50}	Rat ♂	2430 mg/kg	>5000 mg/kg*	7060 mg/kg	>2000 mg/kg*
Acut Derm LD_{50}	GPg	4280 mg/kg+	>3333 mg/kg*	>10 g/kg*	>2000 mg/kg*
21d Cum Irrit	Man	Slight - occlu Neg - open	Neg	-----	Neg
Skin Sensitiz	Man	Neg	Neg	-----	Neg

* The highest level tested.
+ Performed in rabbits.

9. CONCLUSIONS.

a. A mixture of the insect repellents Deet and AI3-37220 in 95 percent alcohol (25:25:50) did not cause primary skin irritation in animals. The mixture was moderately toxic by the oral route but was essentially nontoxic dermally. No photochemical irritation or skin sensitization was demonstrated in animals. In humans, no skin irritation nor sensitization was produced by the repellents' mix.

b. The toxicity of the repellents' mixture did not exceed the toxicities of the individual components. Accordingly, neither additive nor synergistic effects were demonstrated.

10. RECOMMENDATIONS.

a. Based upon the results of toxicity studies in animals, and in humans, it is recommended that repellents mixtures containing up to 25 percent each of Deet and AI3-37220 undergo advanced entomological testing in humans.

b. Based upon the known eye irritation potential of the individual components, it is recommended that mixtures of Deet and AI3-37220 in ethyl alcohol be used with caution around the eyes and mucosa.

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26 July 95
Date

APPENDIX A

REFERENCES

1. Report, Phase 1, Short Term Toxicity, Toxicological Study No. 75-51-YA80-94, Assessment of the Relative Toxicity of Candidate Insect Repellent AI3-37220, 1-(3-Cyclohexen-1-ylcarbonyl)-2-Methylpiperidine, December 1993. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland.

2. Report, Topical Hazard Evaluation Program of Candidate Insect Repellents AI3-37220, 1-(3-Cyclohexen-1-ylcarbonyl)-2-Methylpiperidine and AI3-37224, 1-(3-Cyclohexen-1-ylcarbonyl)-1,2,3,6-Tetrahydropyridine, Study Nos. 75-51-0027-80 and 75-51-0031-80, May 1977 to May 1980. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland.

3. Toxicology Division (TOX), Standing Operating Procedure (SOP) No. 8, Primary Skin Irritation Study in Rabbits, 15 January 1993; with modification, 10 May 1993.

4. TOX SOP No. 12, Photochemical Skin Irritation Study in Rabbits, 12 October 1993.

5. Buehler, E.V., 1965, Delayed Contact Hypersensitivity in the Guinea Pig, *Arch. Dermatol.*, 91:171-177.

6. TOX SOP No. 35, Skin Sensitization in Guinea Pigs, 3 May 1993.

7. TOX SOP No. 17, Approximate Lethal Dose Procedures, 15 January 1993.

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APPENDIX B

PRIMARY SKIN IRRITATION IN RABBITS

Test Substance: 25 percent Deet and 25 percent AI3-37220 in 95 percent ethyl alcohol

Amount Applied: 0.5 mL

Exposure Time: 24 hours; occlusive

Date Started: 1 March 1994

Irritation Scores:

Rabbit No.	Erythema			Edema		
	24 Hour	72 Hour	7 Day	24 Hour	72 Hour	7 Day
176	0	0	0	0	0	0
177	0	0	0	0	0	0
178	0	0	0	0	0	0
180	0	0	0	0	0	0
181	0	0	0	0	0	0
183	0	0	0	0	0	0

Total Score = $\frac{\text{SUM of all 24 and 72 hr scores}}{\text{2 X Number of Animals}}$ = 0.0 EPA Toxicity Category = IV

APPENDIX C

PHOTOCHEMICAL SKIN IRRITATION IN RABBITS

Test Substance: 25 percent Deet and 25 percent AI3-37220 in 95 percent ethyl alcohol

Amount Applied: 0.05 mL

Date Started: 15 February 1994

Irritation Scores:

	UV Irradiated Sites						Nonirradiated Sites					
	Erythema			Edema			Erythema			Edema		
An #	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
187	2	2	1	0	0	0	1	2	1	0	0	0
188	2	2	0	0	0	0	2	2	0	0	0	0
189	2	2	2	1	0	0	2	1	2	0	0	0
190	2	1	0	0	0	0	2	1	1	0	0	0
192	2	1	0	0	0	0	0	1	0	0	0	0
193	2	2	2	1	0	0	2	2	2	1	1	0

Total Erythema	27	24
Edema	2	2
Net Erythema	3	
Edema	0	

Erythema Score (net erythema ÷ no. observations) = 0.16
Edema Score (net edema ÷ no. observations) = 0.00

APPENDIX D

PHOTOCHEMICAL SKIN IRRITATION IN RABBITS
(POSITIVE CONTROL)

Test Substance: Oil of Bergamot (Lot D6066-A); 10 percent in 95 percent ethyl alcohol

Amount Applied: 0.05 mL

Date Started: 15 February 1994

Irritation Scores:

	UV Irradiated Sites						Nonirradiated Sites					
	Erythema			Edema			Erythema			Edema		
An #	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
187	1	1	1	0	0	0	0	0	0	0	0	0
188	1	2	2	0	1	0	0	0	0	0	0	0
189	2	2	2	0	1	1	0	0	0	0	0	0
190	1	1	0	0	0	0	0	0	0	0	0	0
192	1	1	1	0	0	0	1	0	0	0	0	0
193	0	0	1	0	0	0	0	0	0	0	0	0

Total Erythema	20	1
Edema	3	0
Net Erythema	19	
Edema	3	

Erythema Score (net erythema ÷ no. observations) = 1.05
Edema Score (net edema ÷ no. observations) = 0.16

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APPENDIX E

SKIN SENSITIZATION IN GUINEA PIGS

Test Substance: 25 percent Deet and 25 percent AI3-37220 in 95 percent ethyl alcohol

Concentration of Substance for Induction: 5 percent A.I. (w/v) in 80 percent ethyl alcohol

Concentration of Substance for Challenge: 1 percent A.I. (w/v) in 80 percent ethyl alcohol

Date Start Induction: 5 May 1994 **Date Challenged:** 1 June 1994

Irritation Scores:

	Initial Induction				Challenge			
	24 hr		72 hr		24 hr		72 hr	
Animal No.	Eryth	Edema	Eryth	Edema	Eryth	Edema	Eryth	Edema
94.101	1	0	1	0	0	0	0	0
94.102	1	0	0	0	0	0	0	0
94.103	0	0	0	0	0	0	1	0
94.104	2	0	1	0	0	0	0	0
94.105	1	0	0	0	0	0	0	0
94.106	1	0	0	0	0	0	0	0
94.107	2	0	2	0	0	0	0	0
94.108	1	0	1	0	0	0	0	0
94.109	0	0	1	0	1	0	0	0
94.110	1	0	0	0	0	0	0	0
94.111	1	0	1	0	0	0	0	0
94.112	0	0	0	0	0	0	0	0
94.113	2	0	1	0	0	0	0	0

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	Initial Induction				Challenge			
	24 hr		72 hr		24 hr		72 hr	
Animal No.	Eryth	Edema	Eryth	Edema	Eryth	Edema	Eryth	Edema
94.114	1	0	1	0	1	0	1	0
94.115	0	0	0	0	0	0	0	0
94.116	1	0	1	0	0	0	0	0
94.117	1	0	0	0	0	0	0	0
94.118	1	0	0	0	0	0	0	0
94.119	1	0	1	0	0	0	0	0
94.120	1	0	1	0	0	0	0	0
94.121					0	0	0	0
94.122					0	0	0	0
94.123					0	0	0	0
94.124					1	0	0	0
94.125					0	0	0	0
94.126					0	0	0	0
94.127					0	0	0	0
94.128					0	0	0	0
94.129					0	0	0	0
94.130					0	0	0	0

APPENDIX F

ORAL TOXICITY - MALE 25:25

Test Substance: 25 percent Deet and 25 percent AI3-37220 in 95 percent ethyl alcohol

Date of Treatment: 5 May 1994

Route: Oral; gavage

Species: Rat

Sex: Male

Results:

Animal No.	Dose mg/kg*	Effects (minutes)	Recovery minutes
225	2000	lethargy (30)	19 hrs
226	2000	lethargy (30) ataxia (88)	19 hrs 19 hrs
227	2000	lethargy (27)	19 hrs
228	2000	lethargy (26)	19 hrs
229	2000	lethargy (25)	19 hrs

* mg active ingredient/kg

Mortality: 0/5 **24 hrs** 0/5 **48 hrs** 0/5 **72 hrs** 0/5 **14 da**

LD_{50} : >2000 **mg/kg**

APPENDIX G

ORAL TOXICITY - FEMALE 25:25

Test Substance: 25 percent Deet and 25 percent AI3-37220 in 95 percent ethyl alcohol

Date of Treatment: 5 May 1994

Route: Oral; gavage

Species: Rat

Sex: Female

Results:

Animal No.	Dose mg/kg*	Effects (minutes)	Recovery minutes
245	2000	lethargy (27)	19 hrs
246	2000	lethargy (26)	19 hrs
247	2000	lethargy (25)	19 hrs
248	2000	lethargy (23) death (5-19 hrs)	
255	2000	ataxia (60) death (5-19 hrs)	

* mg active ingredient/kg

Mortality: 2/5 **24 hrs** 2/5 **48 hrs** 2/5 **72 hrs** 2/5 **14 da**

Predicted LD_{50} : $\geq$2000 **mg/kg**

Toxicological Study No. 75-51-Y2Z6-95, May 95

APPENDIX H

ORAL TOXICITY - MALE 50:50

Test Substance: 50 percent Deet and 50 percent AI3-37220

Date of Treatment: 5 May 1994

Route: Oral; gavage

Species: Rat

Sex: Male

Results:

Animal No.	Dose mg/kg*	Effects (minutes)	Recovery minutes
230	2000		
231	2000	lethargy (13)	19 hrs
232	2000		
233	2000		
234	2000		

* mg active ingredient/kg

Mortality: 0/5 **24 hrs** 0/5 **48 hrs** 0/5 **72 hrs** 0/5 **14 da**

LD_{50} : >2000 **mg/kg**

APPENDIX I

ORAL TOXICITY - FEMALE 50:50

Test Substance: 50 percent Deet and 50 percent AI3-37220

Route of Treatment: 5 May 1994

Route: Oral; gavage

Species: Rat

Sex: Female

Results:

Animal No.	Dose mg/kg*	Effects (minutes)	Recovery minutes
251	2000		
252	2000		
253	2000		
254	2000		
256	2000	prostrate (21 hr) death (40 hrs)	

* mg active ingredient/kg

Mortality: 0/5 **24 hrs** 1/5 **48 hrs** 1/5 **72 hrs** 1/5 **14 da**

Predicted LD_{50} : $\geq$2000 **mg/kg**

APPENDIX J

DERMAL TOXICITY

Test Substance: 25 percent Deet and 25 percent AI3-37220 in 95 percent ethyl alcohol

Date of Treatment: 1 June 1994

Route: Dermal; 24 hr occlusive

Species: Guinea pig

Sex: Either

Results:

Animal No.	Dose mg/kg*	Effects (minutes)	Recovery minutes
94.191	2000	None	
94.192	2000	None	
94.193	2000	None	
94.194	2000	None	
94.195	2000	None	

* mg active ingredient/kg

Mortality: 0/5 **24 hrs** 0/5 **48 hrs** 0/5 **72 hrs** 0/5 **14 da**

LD_{50} : >2000 **mg/kg**

APPENDIX K

21-DAY CUMULATIVE IRRITANCY - HUMANS

Formulation Summary:

Site 1 25% Deet and 25% AI3-37220 in ethanol (95%)
2 12.5% Deet and 12.5% AI3-37220 in ethanol (95%)
3 6.25% Deet and 6.25% AI3-37220 in ethanol (95%)
4 3.125% Deet and 3.125% AI3-37220 in ethanol (95%)
5 25% Deet in ethanol (95%)
6 Ethanol (95%)
7 25% AI3-37220 in ethanol (95%)

Cumulative Irritancy Scores*:

Subject	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
1 SG	4	3	3	3	6	0	6
2 JD	0	0	0	0	0	0	0
3 PM	5	5	2.5	1	3	0	3
4 MP	0	0	0	0	0	0	0
5 JS	0	0	0	0	0	4	0
6 BA	5.5	5.5	6	0	7.5	3	8
7 PM	0	0	0	0	0	0	0
8 MS	1.5	1.5	0	0	3	0	4.5
9 NB	Cancel						
10 DD	0	0	0	0	0	0	0
11 RD	0	0	0	0	0	0	0
TOTAL	16	15	11.5	4	19.5	7	21.5

* Total of 15 observations per subject.

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APPENDIX L

SKIN SENSITIZATION STUDY - HUMANS

Formulation Summary:

Site 1 3.125% Deet and 3.125% AI3-37220 in ethanol (95%)
2 25% Deet and 25% AI3-37220 in ethanol (95%)
3 100% AI3-37220

	Induction Score*			**Challenge Score+**		
	Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
TOTAL	7	37	36	0.5	2.5	0.5
STD DEV	0.35	0.79	0.93	0.04	0.11	0.04

* Cumulative score of 200 subjects; 9 observations per subject.
+ Cumulative score of 200 subjects; 2 observations per subject.

APPENDIX M

ARCHIVES AND PERSONNEL

1. ARCHIVES.

a. All raw and summary data, and the final report regarding the toxicological testing of mixtures of Deet and AI3-37220 are maintained in the administrative office of the Toxicology Programs, USACHPPM (Prov), under file 85-48-2324. Laboratory notebook number 162 which contains an account of the day-to-day study operations is also filed under study number 85-48-2324. A copy of the approved study protocol, the pertinent SOPs, and an account of any protocol modifications and/or SOP deviations are also filed under 85-48-2324.

b. Records on animal receipt, diet and environmental parameters are maintained in Room 3014 (Toxicology), Bldg E2100, USACHPPM (Prov).

c. The present study used the laboratory project ID 75-51-Y2Z6-94 which appears on all study data. This number, however, was changed to 85-48-2324 for administrative reasons after the completion of testing.

2. PERSONNEL. The following personnel from the Toxicology Programs participated in the studies reported herein:

Hubert L. Snodgrass	Theresa L. Hanna	Richard A. Arnold
John G. Harvey	Patricia A. Beall	Frank Miskena
John T. Houpt	Robert L. Sunderland	